

Figure S1. HCMV Pr activity and dimerization, related to Figures 1 and 2. a) Catalytic efficiency of HCMV Pr (red curve), HCMV C161A (blue curve), and HCMV C-A (black curve), determined by monitoring *in vitro* substrate cleavage. Data are plotted in technical triplicate and are reported as the mean ± SD. b) Location of L222D on the HCMV Pr structure (PDB code: 1NJU). One monomeric chain is blue, the second is greencyan. C161 is yellow, the catalytic triad is red. L222 on each monomer chain is magenta. c) Monomer/dimer equilibrium of varying concentrations of HCMV C-A determined by SEC. Top: full HCMV C-A SEC trace, with the HCMV C-A monomer/dimer boxed. Bottom left: Zoom-in of the HCMV C-A monomer/dimer. Bottom middle: HCMV C-A L222D under the same assay conditions as HCMV C-A. Bottom right: Overlay of HCMV C-A and HCMV C-A L222D. d) ^1H - ^{15}N HSQC spectrum of HCMV Δ221 (black), HCMV L222D (red) and HCMV C-A L222D (blue).

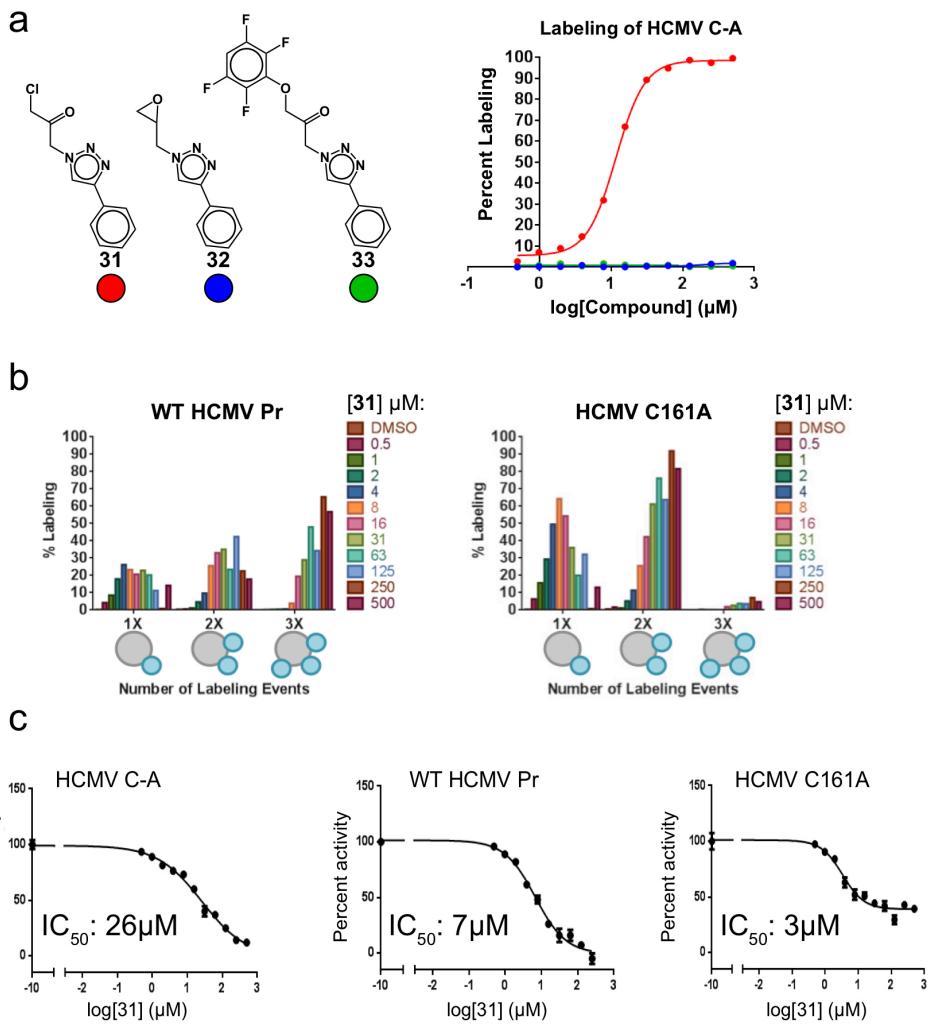


Figure S2. Conversion of disulfide inhibitors to irreversible electrophiles, related to Table 1. a) Labeling of HCMV C-A with 4-phenyl-1,2,3-triazole electrophiles bearing a chloromethyl ketone (**31**), epoxide (**32**) or tetrafluorophenoxy methyl ketone (**33**). Labeling was determined by intact protein LC/MS at one hour compound incubation and are collected from a single replicate. b) Labeling of WT HCMV Pr or HCMV C161A with varying concentrations of **31** at one hour incubation. Masses corresponding to apo protein and protein + one, two or three **31** molecules were observed and used to quantify percent labeling for each adduct type. Data over all concentrations of **31** are plotted in bar graphs from a single replicate. c) *In vitro* inhibition of HCMV C-A, WT HCMV Pr, or HCMV C161A at one hour incubation with **31**. Data are plotted in triplicate as mean \pm SD. Note, IC_{50} values for HCMV C-A and WT HCMV Pr are slightly different than Table 1 because they were determined in the absence of BME.

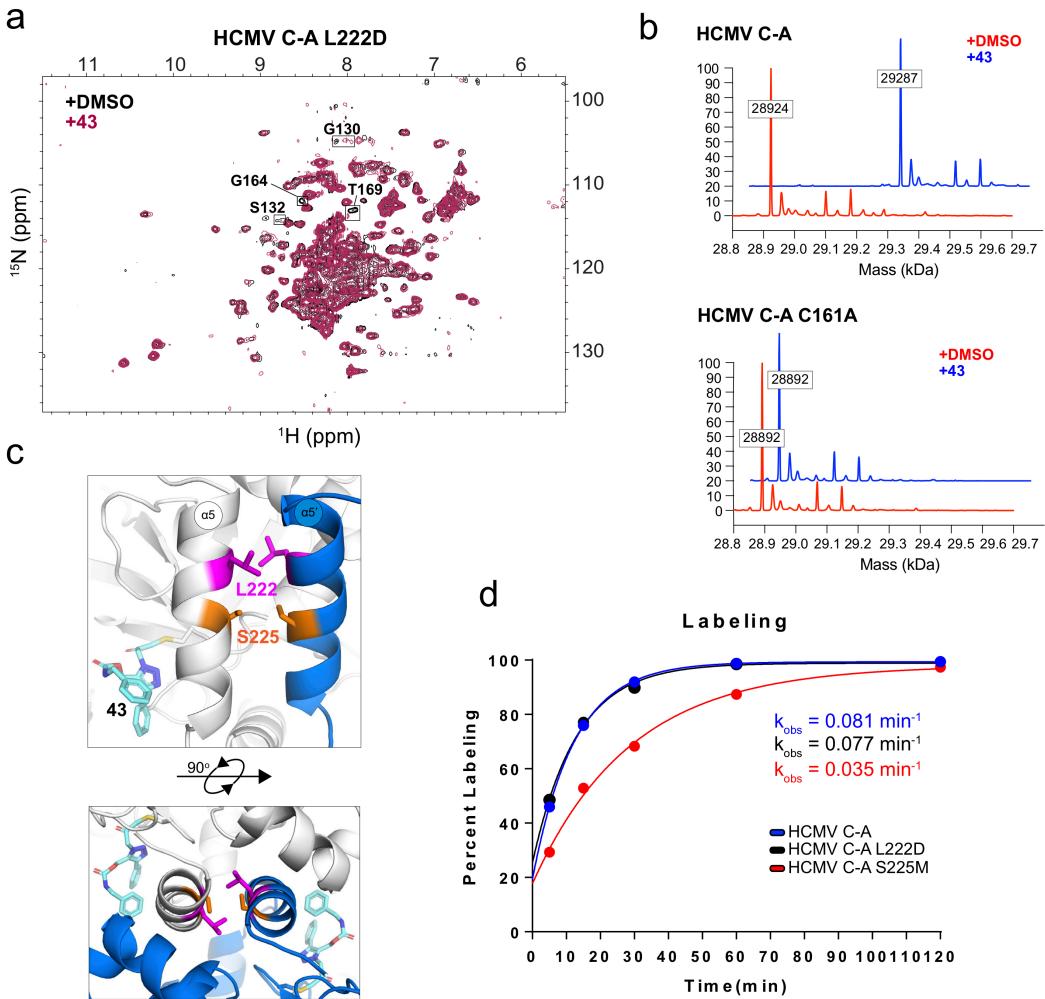


Figure S3. Inhibitor 43 mechanism of action, related to Figures 3 and 4. a) ^1H - ^{15}N HSQC of HCMV C-A L222D plus d_6 -DMSO (black spectrum) or **43** (maroon). Four residues (G130, S132, G164, T169) were significantly perturbed (see Methods and Table S4). b) LC/MS analysis of **43** labeling of HCMV C-A or HCMV C-A C161A when incubated overnight in Tris buffer. The expected mass of HCMV C-A is 28,922 Da and the observed is 28,924 Da. The expected mass of HCMV C-A C161A is 28,890 Da and the observed is 28,892 Da. The expected mass adduct for **43** is + 363 Da. The three minor peaks in each spectrum are a M+35 Da, M+177 Da and M+257 Da peaks that are consistent across all samples and likely reflect adduct formation with buffer components. c) The dimer interfacial helix $\alpha 5$ is shown in white (monomer 1, chain A) and blue (monomer 2, chain B). L222 on each helix is magenta and S225 is orange. d) Time-dependent **43** labeling of HCMV C-A, HCMV C-A L222D and HCMV C-A S225M without DTT quenching (see Methods). Data are plotted from a single replicate.

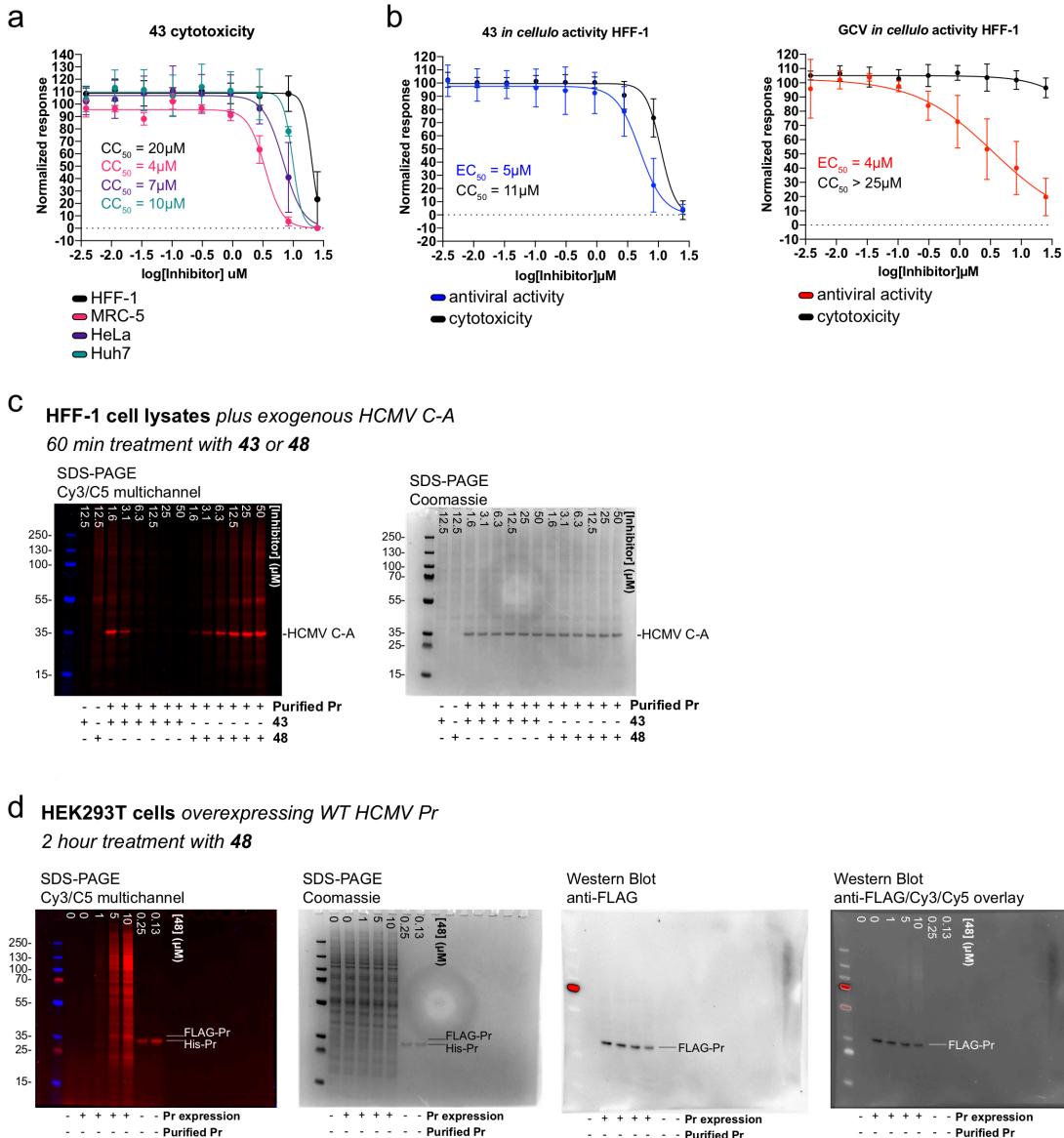


Figure S4. Compound 43 cellular activity, related to Figure 5. a) Cytotoxicity of **43** in HFF-1, MRC-5, Huh7 and HeLa cells. Data are plotted in triplicate as the mean \pm SD. b) HCMV infectivity of HFF-1 cells after three days treatment with **43** or ganciclovir. Cytotoxicity was simultaneously monitored. Data are plotted from five replicates (a mixture of technical and biological replicates) as the mean \pm SD. c) In-gel fluorescence assay monitoring **43** competition for cysteine reactivity with a general alkyne maleimide probe or direct detection of **48** labeling. A serial dilution of **43** or **48** was introduced to HFF-1 cell lysates supplemented with purified HCMV C-A and incubated for 60 min. Left: Cy3/Cy5 fluorescence; right: Coomassie. d) HEK293T cells overexpressing FLAG-tagged WT HCMV Pr treated with increasing concentrations of **48** for two hours. Purified His-tagged WT HCMV Pr labeled with **48** in buffer is included as a control, as well as HEK293T lysate lacking the HCMV Pr plasmid. Samples were used to run two gels simultaneously, one of which was imaged for Cy3/Cy5 fluorescence, the other Coomassie stained. The gel used for Cy3/Cy5 imaging was also used in anti-FLAG western blot. The far right is an overlay of the Cy3/Cy5 image of the membrane with the antibody signal.

Table S1. Disulfide tethering screening hits, related to Figure 1. Disulfide tethering screening hits against HCMV C-A. Full chemical structures and percent labeling in the screening assay are shown. The IC₅₀ at one hour incubation with HCMV C-A is listed for tested hits, a dash indicates that IC₅₀ was not determined.

Structure	Hit Number	%Labeled	IC ₅₀ (μM)	Structure	Hit Number	%Labeled	IC ₅₀ (μM)
	1	81.5	---		16	67.0	41
	2	54.8	---		17	62.7	168
	3	65.9	---		18	53.9	92
	4	73.2	>250		19	59.3	151
	5	79.2	194		20	54.6	No Inhibition
	6	53.7	---		21	52.5	>250
	7	61.9	>250		22	58.4	No Inhibition
	8	52.9	---		23	76.9	86
	9	53.2	>250		24	83.4	76
	10	57.4	>250		25	77.3	---
	11	63.2	>250		26	68.4	---
	12	66.7	>250		27	79.8	334
	13	70.3	246		28	67.0	---
	14	82.0	---		29	52.6	217
	15	73.1	144		30	52.9	181

Table S2. ^1H - ^{15}N resonances and peak volumes for HCMV C-A L222D + disulfide tethered inhibitors, related to Figure 2. N-H ^{15}N are amide backbones, Nε1-H1 are tryptophan indole sidechains, Nε2-H2 are asparagine/glutamine amide sidechains. A single asterisk indicates a second amide sidechain proton. A double asterisk indicates the same proton, but in a secondary conformation.

HCMV C-A L222D		DMSO	15										
Residue	#	Atom	^{15}N (ppm)	^1H (ppm)	Signal to Noise	Peak Volume	Normalized Peak Volume	^{15}N (ppm)	^1H (ppm)	Peak Volume	Normalized Peak Volume	CSP (ppm)	$V_{\text{inhibitor}}/V_{\text{DMSO}}$
Ala	12	N-HN	129.222	8.035	48	3.86E+08	0.767	129.238	8.035	5.88E+08	0.982	0.00	1.28
Tyr	15	N-HN	124.667	8.858	16	3.59E+08	0.714	124.49	8.87	5.42E+08	0.905	0.02	1.27
Gly	17	N-HN	115.173	9.164	14	1.88E+08	0.374	115.115	9.172	2.27E+08	0.379	0.01	1.01
Gly	18	N-HN	104.979	6.942	20	2.91E+08	0.579	105.037	6.946	2.80E+08	0.467	0.01	0.81
Phe	19	N-HN	116.413	8.957	11	2.29E+08	0.455	116.375	8.919	2.36E+08	0.394	0.04	0.87
Ala	21	N-HN	116.056	7.622	17	1.64E+08	0.326	116.061	7.623	3.28E+08	0.548	0.00	1.68
Val	38	N-HN	120.953	7.106	21	4.26E+08	0.847	120.993	7.094	3.77E+08	0.629	0.01	0.74
Glu	40	N-HN	117.234	8.755	15	1.39E+08	0.276	117.202	8.741	1.66E+08	0.277	0.01	1.00
Trp	42	Nε1-Hε1	129.047	10.207	50	4.35E+08	0.865	128.966	10.201	5.77E+08	0.963	0.01	1.11
Gln	46	Nε2-Hε2*	111.838	7.153	28	1.72E+08	0.342	111.766	7.188	3.81E+08	0.636	0.04	1.86
Gly	49	N-HN	111.837	6.571	43	3.44E+08	0.684	111.805	6.586	3.56E+08	0.594	0.02	0.87
His	63	N-HN	109.25	8.446	34	3.66E+08	0.728	109.201	8.437	3.98E+08	0.664	0.01	0.91
Thr	66	N-HN	105.99	6.67	9	1.42E+08	0.282	106.46	6.696	6.29E+08	1.050	0.05	3.72
Ala	67	N-HN	110.159	8.685	17	1.81E+08	0.360	110.079	8.666	1.61E+08	0.269	0.02	0.75
Gly	70	N-HN	129.824	7.919	22	3.13E+08	0.622	129.855	7.92	4.27E+08	0.713	0.00	1.15
Ala	73	N-HN	109.318	7.597	19	1.88E+08	0.374	109.343	7.593	2.55E+08	0.426	0.00	1.14
Arg	79	N-HN	132.429	8.968	10	2.16E+08	0.429	132.346	8.994	4.67E+07	0.078	0.03	0.18
Phe	83	N-HN	121.472	8.72	22	2.96E+08	0.588	121.497	8.738	4.63E+08	0.773	0.02	1.31
Gly	86	N-HN	130.606	9.058	13	2.38E+08	0.473	130.739	9.065	3.15E+08	0.526	0.02	1.11
Ile	96	N-HN	113.517	10.325	12	1.78E+08	0.354	113.612	10.319	2.45E+08	0.409	0.01	1.16
Ser	104	N-HN	122.28	7.571	14	3.52E+08	0.700	122.31	7.57	4.51E+08	0.753	0.00	1.08
Ser	125	N-HN	114.701	7.62	39	4.04E+08	0.803	114.686	7.617	5.83E+08	0.973	0.00	1.21
Ser	134	N-HN	111.844	7.815	17	1.18E+08	0.235	111.889	7.817	1.16E+08	0.194	0.00	0.83
Gly	126	N-HN	103.712	7.349	20	2.32E+08	0.461	103.636	7.348	2.82E+08	0.471	0.01	1.02
Ser	127	N-HN	115.407	7.412	17	1.80E+08	0.358	115.438	7.43	2.19E+08	0.366	0.01	1.02
Gly	130	N-HN	104.774	8.128	9	3.25E+08	0.646	N/A	N/A	0.000	N/A	0.00	0.83
Leu	133	N-HN	128.306	9.111	7	6.50E+07	0.129	129.479	9.133	4.70E+08	0.785	0.12	6.07
Ser	134	N-HN	116.052	7.695	21	1.98E+08	0.394	116.055	7.694	1.49E+08	0.249	0.00	0.63
Thr	145	N-HN	112.043	8.015	95	4.70E+08	0.934	111.975	8.011	6.07E+08	1.013	0.01	1.08
Phe	155	N-HN	116.329	7.07	21	2.28E+08	0.453	116.446	7.066	4.41E+08	0.736	0.01	1.62
Ala	159	N-HN	128.755	9.233	9	1.47E+08	0.292	128.962	9.26	3.12E+08	0.521	0.03	1.78
Gly	168	N-HN	109.502	8.601	18	2.21E+08	0.439	109.596	8.54	1.81E+08	0.302	0.06	0.69
Gly	169	N-HN	113.011	7.938	22	1.94E+08	0.386	113.099	7.916	6.19E+07	0.103	0.02	0.27
Thr	174	N-HN	103.821	8.968	17	2.16E+08	0.429	103.832	8.954	2.55E+08	0.426	0.01	0.99
Arg	175	N-HN	118.277	8.87	8	7.42E+07	0.148	118.233	8.862	1.18E+08	0.197	0.01	1.34
Trp	179	Nε1-Hε1	130.417	10.242	68	5.03E+08	1.000	130.422	10.24	5.99E+08	1.000	0.00	1.00
Val	180	N-HN	116.098	7.798	22	2.68E+08	0.533	116.252	7.81	3.78E+07	0.063	0.02	0.12
Thr	181	N-HN	107.653	7.709	22	3.06E+08	0.608	107.598	7.711	1.99E+08	0.332	0.01	0.55
Phe	184	N-HN	113.85	7.04	25	2.70E+08	0.537	118.477	7.044	3.45E+08	0.576	0.00	1.07
Asp	186	N-HN	114.603	8.683	34	3.82E+08	0.759	114.609	8.683	3.23E+08	0.539	0.00	0.71
Thr	188	N-HN	115.266	9.522	16	1.69E+08	0.336	115.303	9.532	2.60E+08	0.434	0.01	1.29
Gly	194	N-HN	108.563	7.876	30	2.99E+08	0.594	108.551	8.875	4.73E+08	0.790	0.00	1.33
Trp	199	Nε1-Hε1	131.27	10.588	40	3.40E+08	0.594	131.241	10.585	4.73E+08	0.790	0.00	1.17
Gln	200	Nε2-Hε2	110.67	7.208	39	3.11E+08	0.618	110.658	7.204	5.04E+08	0.841	0.00	1.36
Gly	211	N-HN	110.379	8.084	77	5.65E+08	1.123	110.365	8.084	7.81E+08	1.304	0.00	1.16
Arg	215	N-HN	127.157	7.859	11	1.04E+08	0.207	127.199	7.877	1.50E+08	0.250	0.02	1.21

HCMV-C-AL222D		18		23										
Residue	#	Atom	¹⁵ N (ppm)	¹ H (ppm)	Peak Volume	Normalized Peak Volume	CSP (ppm)	V _{inhibitor} /V _{DMSO}	¹⁵ N (ppm)	¹ H (ppm)	Peak Volume	Normalized Peak Volume	CSP (ppm)	V _{inhibitor} /V _{DMSO}
Ala	12	N-HN	129.206	8.034	3.74E+08	0.822	0.00	1.07	129.221	8.035	3.60E+08	0.813	0.00	1.06
Tyr	15	N-HN	124.421	8.878	3.98E+08	0.875	0.03	1.23	124.525	8.875	4.44E+08	1.002	0.02	1.40
Gly	17	N-HN	115.013	9.16	1.34E+08	0.295	0.02	0.79	115.046	9.161	1.27E+08	0.287	0.01	0.77
Gly	18	N-HN	105.012	6.941	2.69E+08	0.591	0.00	1.02	105.05	6.947	1.99E+08	0.449	0.01	0.78
Phe	19	N-HN	116.348	8.922	9.81E+07	0.216	0.04	0.47	116.296	8.926	1.30E+08	0.293	0.03	0.64
Ala	21	N-HN	115.987	7.635	2.50E+08	0.549	0.01	1.69	116.048	7.64	3.41E+08	0.770	0.02	2.36
Val	38	N-HN	120.897	7.08	3.44E+08	0.756	0.03	0.89	120.954	7.091	3.14E+08	0.709	0.02	0.84
Glu	40	N-HN	117.204	8.744	9.79E+07	0.215	0.01	0.78	117.173	8.743	1.26E+08	0.284	0.01	1.03
Trp	42	Nε1-He1	128.958	10.198	3.92E+08	0.862	0.01	1.00	128.964	10.199	4.53E+08	1.023	0.01	1.18
Gln	46	Nε2-He2	111.73	7.19	2.49E+08	0.547	0.04	1.60	111.724	7.188	2.53E+08	0.571	0.04	1.67
Gly	49	N-HN	111.754	6.588	3.57E+08	0.785	0.02	1.15	N/A	N/A	0.00	N/A	0.00	N/A
His	63	N-HN	109.155	8.435	2.83E+08	0.622	0.01	0.85	109.213	8.444	3.62E+08	0.817	0.00	1.12
Thr	66	N-HN	106.462	6.709	6.55E+07	0.144	0.06	0.51	106.517	6.699	4.36E+07	0.998	0.06	0.35
Ala	67	N-HN	129.883	7.921	3.67E+08	0.807	0.01	1.30	129.888	7.924	2.56E+08	0.578	0.01	0.93
Gly	70	N-HN	109.365	7.591	2.18E+08	0.479	0.01	1.28	109.368	7.6	1.58E+08	0.357	0.01	0.95
Ala	73	N-HN	132.458	8.979	2.52E+08	0.554	0.01	1.29	132.349	8.967	7.31E+07	0.165	0.01	0.38
Arg	79	N-HN	121.444	8.73	3.00E+08	0.659	0.01	1.12	121.512	8.739	2.54E+08	0.573	0.02	0.97
Phe	83	N-HN	130.676	9.074	1.91E+08	0.420	0.02	0.89	130.627	9.073	1.57E+08	0.354	0.02	0.75
Gly	86	N-HN	113.5	10.333	1.79E+08	0.393	0.01	1.11	113.51	10.314	1.65E+08	0.372	0.01	1.05
Ile	96	N-HN	122.292	7.577	2.76E+08	0.607	0.01	0.87	122.351	7.585	4.05E+08	0.914	0.02	1.31
Ser	104	N-HN	114.687	7.62	4.08E+08	0.897	0.00	1.12	114.69	7.616	3.70E+08	0.835	0.00	1.04
Ser	125	N-HN	111.81	7.822	1.31E+08	0.288	0.01	1.23	111.824	7.822	9.12E+07	0.206	0.01	0.88
Gly	126	N-HN	103.634	7.352	1.98E+08	0.435	0.01	0.94	103.568	7.35	2.31E+08	0.521	0.01	1.13
Ser	127	N-HN	115.547	7.442	1.50E+08	0.330	0.03	0.92	115.505	7.433	1.76E+08	0.397	0.02	1.11
Gly	130	N-HN	N/A	0.000	N/A	0.00	N/A	N/A	N/A	N/A	0.000	N/A	0.00	N/A
Leu	133	N-HN	129.073	9.159	2.82E+08	0.620	0.09	4.80	129.436	9.144	7.95E+07	0.179	0.12	1.39
Ser	134	N-HN	116.066	7.701	1.48E+08	0.325	0.01	0.83	116.068	7.695	1.30E+08	0.293	0.00	0.75
Thr	145	N-HN	111.983	8.015	4.32E+08	0.949	0.01	1.02	111.979	8.016	4.40E+08	0.993	0.01	1.06
Phe	155	N-HN	116.446	7.076	2.50E+08	0.549	0.01	1.21	116.452	7.078	2.87E+08	0.648	0.01	1.43
Ala	159	N-HN	128.835	9.233	9.28E+07	0.204	0.01	0.70	128.972	9.317	1.60E+08	0.361	0.09	1.24
Gly	168	N-HN	109.443	8.543	1.16E+08	0.255	0.06	0.58	109.576	8.535	2.11E+08	0.476	0.07	1.08
Thr	169	N-HN	113.21	7.978	2.02E+07	0.044	0.04	0.12	113.62	8.002	7.28E+08	1.643	0.09	4.26
Gly	174	N-HN	103.823	8.961	1.23E+08	0.270	0.01	0.63	103.826	8.951	1.42E+08	0.321	0.02	0.75
Arg	175	N-HN	N/A	0.000	N/A	0.00	N/A	N/A	N/A	N/A	0.000	N/A	0.00	N/A
Trp	179	Nε1-He1	130.426	10.245	4.55E+08	1.000	0.00	1.00	130.441	10.245	4.43E+08	1.000	0.00	1.00
Val	180	N-HN	116.039	7.749	2.67E+07	0.059	0.05	0.11	116.069	7.727	4.71E+07	0.106	0.07	0.20
Thr	181	N-HN	107.598	7.706	1.68E+08	0.369	0.01	0.61	107.643	7.711	9.45E+07	0.213	0.00	0.35
Phe	184	N-HN	118.529	7.042	2.77E+08	0.609	0.00	1.13	118.515	7.046	2.88E+08	0.650	0.01	1.21
Asp	186	N-HN	114.62	8.686	1.97E+08	0.433	0.00	0.57	114.616	8.683	2.56E+08	0.578	0.00	0.76
Thr	188	N-HN	115.337	9.529	1.44E+08	0.316	0.01	0.94	115.226	9.528	1.36E+08	0.307	0.01	0.91
Gly	194	N-HN	108.596	7.876	1.44E+08	0.316	0.00	0.53	108.592	7.88	2.57E+08	0.580	0.00	0.98
Trp	199	Nε1-He1	131.253	10.588	2.81E+08	0.618	0.00	0.91	131.248	10.584	4.26E+08	0.962	0.00	1.42
Gln	200	N-HN	110.64	7.206	3.68E+08	0.809	0.00	1.31	110.664	7.206	3.62E+08	0.817	0.00	1.32
Gly	211	Nε2-He2	110.384	5.21E+08	1.145	0.00	1.02	110.381	5.21E+08	1.300	0.00	1.16	1.07	
Arg	215	N-HN	127.205	7.871	1.28E+08	0.281	0.01	1.36	127.172	7.865	9.76E+07	0.220	0.01	1.07

HCMV C-A L222D

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Residue	#	Atom	¹⁵ N (ppm)	¹ H (ppm)	Peak Volume	Normalized Peak Volume	CSP (ppm)	V _{Inhibitor} /V _{so}
Ala	12	N-HN	129.244	8.034	3.08E+08	0.688	0.00	0.90
Tyr	15	N-HN	124.484	8.869	5.23E+08	1.167	0.02	1.64
Gly	17	N-HN	115.045	9.152	2.63E+08	0.587	0.02	1.57
Gly	18	N-HN	105.072	6.949	2.11E+08	0.471	0.01	0.81
Phe	19	N-HN	116.304	8.928	1.78E+08	0.397	0.03	0.87
Ala	21	N-HN	116.023	7.626	1.97E+08	0.440	0.01	1.35
Val	38	N-HN	120.951	7.095	3.17E+08	0.708	0.01	0.84
Glu	40	N-HN	117.167	8.742	1.82E+08	0.406	0.01	1.47
Trp	42	Nε1-Hε1	128.952	10.202	3.99E+08	0.891	0.01	1.03
Gln	46	Nε2-Hε2	111.753	7.186	2.45E+08	0.547	0.03	1.60
		Nε2-Hε2*	111.778	6.585	2.69E+08	0.600	0.02	0.88
Gly	49	N-HN	109.186	8.435	3.02E+08	0.674	0.01	0.93
His	63	N-HN	106.688	6.705	1.74E+08	0.388	0.08	1.38
Thr	66	N-HN	110.071	8.676	1.48E+08	0.330	0.01	0.92
Ala	67	N-HN	129.837	7.916	3.59E+08	0.801	0.00	1.29
Gly	70	N-HN	109.334	7.597	1.89E+08	0.422	0.00	1.13
Ala	73	N-HN	132.194	8.954	3.01E+08	0.672	0.03	1.56
Arg	79	N-HN	121.462	8.745	3.05E+08	0.681	0.03	1.16
Phe	83	N-HN	130.69	9.069	2.61E+08	0.583	0.01	1.23
Gly	86	N-HN	133.55	10.329	7.22E+07	0.161	0.01	0.46
Ile	96	N-HN	122.34	7.583	2.55E+08	0.569	0.01	0.81
Ser	104	N-HN	114.682	7.62	3.33E+08	0.743	0.00	0.93
Ser	125	N-HN	111.851	7.816	1.39E+08	0.310	0.00	1.32
Gly	126	N/A	N/A	N/A	0.000	N/A	0.00	0.00
Ser	127	N-HN	115.497	7.429	2.07E+08	0.462	0.01	1.29
Gly	130	N-HN	N/A	N/A	0.000	N/A	0.00	0.00
Leu	133	N-HN	129.383	9.147	9.20E+07	0.205	0.11	1.59
Ser	134	N-HN	116.051	7.695	6.86E+07	0.153	0.00	0.39
Thr	145	N-HN	111.974	8.012	4.98E+08	1.112	0.01	1.19
Phe	155	N-HN	116.447	7.079	2.47E+08	0.551	0.01	1.22
Ala	159	N-HN	128.932	9.275	1.90E+08	0.424	0.05	1.45
Gly	168	N-HN	109.625	8.546	1.82E+08	0.406	0.06	0.92
Thr	169	N-HN	113.666	8.012	4.19E+08	0.935	0.10	2.42
Gly	174	N-HN	103.837	8.958	1.74E+08	0.388	0.01	0.90
Arg	175	N-HN	N/A	N/A	0.000	N/A	0.00	0.00
Trp	179	Nε1-Hε1	130.434	10.244	4.48E+08	1.000	0.00	1.00
Val	180	N-HN	115.96	7.794	4.73E+06	0.011	0.01	0.02
Thr	181	N-HN	107.613	7.709	1.85E+08	0.413	0.00	0.68
Phe	184	N-HN	118.532	7.048	3.01E+08	0.672	0.01	1.25
Asp	186	N-HN	114.603	8.686	2.80E+08	0.625	0.00	0.82
Thr	188	N-HN	115.289	9.53	2.21E+08	0.493	0.01	1.47
Gly	194	N-HN	108.56	7.884	1.95E+08	0.435	0.01	0.73
Trp	199	Nε1-Hε1	131.229	10.585	2.61E+08	0.583	0.01	0.86
Gln	200	Nε2-Hε2	110.708	7.206	3.37E+08	0.752	0.00	1.22
Gly	211	N-HN	110.377	8.085	5.36E+08	1.196	0.00	1.07
Arg	215	N-HN	127.214	7.868	8.13E+07	0.181	0.01	0.88

Table S3. Inhibition kinetics of chloromethyl ketones, related to Table 1. Several CMK inhibitors were tested for K_I and k_{inact} values against HCMV C-A (left) and **43** was also tested against HCMV C-A S225M (right). Inhibition was monitored over time at varying concentration of inhibitors. These curves were fit in GraphPad prism from data collected in triplicate (HCMV C-A) or duplicate (HCMV C-A S225M) to determine k_{obs} values. k_{obs} was plotted against inhibitor concentration then fit in Prism to determine K_I and k_{inact} values.

HCMV C-A							HCMV C-A S225M						
Compound number	[Inhibitor] (μM)	K_{obs} min $^{-1}$	K_I (μM)	k_{inact} (min $^{-1}$)	t $_{1/2}$ (min)	k_{inact}/K_I (μM^{-1} min $^{-1}$)	Compound number	[Inhibitor] (μM)	K_{obs} min $^{-1}$	K_I (μM)	k_{inact} (min $^{-1}$)	t $_{1/2}$ (min)	k_{inact}/K_I (μM^{-1} min $^{-1}$)
31	0 0.5 1 2 4 8 16 31 63 125 250 500	9.95E-13 0.0003 0.0006 0.0016 0.0034 0.0065 0.0093 0.0104 0.0140 0.0213 0.0249 0.0237	38	0.026	26	7.0E-04	43	0 1 2.5 5 10 20 40 80	4.35E-05 0.0012 0.0020 0.0030 0.0042 0.0061 0.0099 0.0084	13	0.011	64	8.5E-04
43	0 0.25 0.5 1 2 4 8 16 31 63	4.97E-11 0.0008 0.0012 0.0021 0.0035 0.0071 0.0121 0.0161 0.0191 0.0204	8	0.024	29	2.8E-03							
44	0 0.5 1 2 4 8 16 31 63 125 250 500	1.33E-13 0.0001 0.0006 0.0010 0.0023 0.0042 0.0081 0.0117 0.0155 0.0170 0.0203 0.0209	29	0.022	31	7.6E-04							
45	0 0.5 1 2 4 8 16 31 63 125 250 500	8.69E-11 8.04E-05 0.0004 0.0006 0.0016 0.0036 0.0068 0.0114 0.0150 0.0174 0.0182 0.0171	26	0.020	35	7.5E-04							
47	0 0.5 1 2 4 8 16 31 63 125 250 500	9.15E-11 0.0004 0.0006 0.0014 0.0024 0.0037 0.0065 0.0090 0.0121 0.0152 0.0159 0.0127	21	0.016	44	7.5E-04							

Table S4. ^1H - ^{15}N resonances and peak volumes for HCMV C-A L222D + 43, related to Figure 3. The resonances of HCMV C-A L222D +DMSO or +43 were determined as described for disulfide tethered inhibitors. See Table S2 and Methods.

HCMV CA C161 L222D			DMSO					43						
Residue	#	Atom	^{15}N (ppm)	^1H (ppm)	Signal to Noise	Peak Volume	Normalized Peak Volume	^{15}N (ppm)	^1H (ppm)	Peak Volume	Normalized Peak Volume	CSP (ppm)	$V_{\text{Inhibitor}}/V_{\text{DMSO}}$	
Ala	12	N-HN	129.288	8.037	35	6.82E+07	0.669	129.272	8.032	1.63E+08	0.776	0.01	1.16	
Tyr	15	N-HN	124.584	8.853	14	3.99E+07	0.391	124.55	8.856	1.15E+08	0.548	0.00	1.40	
Gly	17	N-HN	115.2	9.179	9	3.12E+07	0.306	115.23	9.164	4.46E+07	0.212	0.02	0.69	
Gly	18	N-HN	104.953	6.936	12	5.27E+07	0.517	105.048	6.954	1.08E+08	0.514	0.02	1.00	
Phe	19	N-HN	116.318	8.908	9	4.17E+07	0.409	116.286	8.904	1.58E+08	0.752	0.01	1.84	
Ala	21	N-HN	116.055	7.625	12	2.21E+07	0.217	115.918	7.612	6.86E+07	0.327	0.02	1.51	
Val	38	N-HN	120.983	7.091	18	3.77E+07	0.370	120.945	7.077	9.69E+07	0.461	0.01	1.25	
Glu	40	N-HN	117.204	8.746	15	6.87E+07	0.674	117.255	8.747	9.94E+07	0.473	0.01	0.70	
Trp	42	N ε 1-H ε 1	129.069	10.199	36	8.41E+07	0.825	129.048	10.19	1.68E+08	0.800	0.01	0.97	
Gln	46	N ε 2-H ε 2	111.804	7.153	22	4.72E+07	0.463	111.833	7.16	5.77E+07	0.275	0.01	0.59	
		N ε 2-H ε 2'	111.864	6.574	33	1.12E+08	1.098	111.804	6.573	8.82E+07	0.420	0.01	0.38	
Gly	49	N-HN	109.353	8.442	30	7.10E+07	0.696	109.357	8.449	2.46E+08	1.171	0.01	1.68	
His	63	N-HN	106.044	6.687	8	2.71E+07	0.266	106.215	6.7	4.66E+07	0.222	0.02	0.84	
Thr	66	N-HN	110.164	8.68	17	4.83E+07	0.474	110.172	8.674	8.06E+07	0.384	0.01	0.81	
Ala	67	N-HN	129.832	7.911	18	7.14E+07	0.700	129.815	7.909	1.62E+08	0.771	0.00	1.10	
Gly	70	N-HN	109.313	7.603	16	3.40E+07	0.333	109.375	7.59	1.02E+08	0.486	0.01	1.46	
Arg	79	N-HN	121.469	8.73	15	3.62E+07	0.355	121.516	8.73	9.67E+07	0.460	0.00	1.30	
Phe	83	N-HN	130.644	9.065	12	3.54E+07	0.347	130.672	9.065	7.62E+07	0.363	0.00	1.05	
Gly	86	N-HN	113.536	10.327	11	2.40E+07	0.235	113.476	10.321	9.86E+07	0.470	0.01	2.00	
Ile	96	N-HN	122.207	7.57	11	2.59E+07	0.254	122.254	7.568	2.63E+07	0.125	0.01	0.49	
Ser	104	N-HN	114.748	7.623	23	5.07E+07	0.497	114.725	7.62	1.44E+08	0.686	0.00	1.38	
Ser	125	N-HN	111.896	7.809	13	1.96E+07	0.192	111.852	7.809	9.17E+06	0.044	0.00	0.23	
Gly	126	N-HN	103.709	7.36	16	4.23E+07	0.415	103.73	7.343	5.98E+07	0.285	0.02	0.69	
Ser	127	N-HN	115.431	7.416	13	3.71E+07	0.364	115.435	7.429	8.57E+07	0.408	0.01	1.12	
Gly	130	N-HN	104.845	8.134	7	1.64E+07	0.161	104.646	7.869	5.99E+07	0.285	0.27	1.77	
Ser	132	N-HN	114.273	8.811	7	2.78E+07	0.273	N/A		0.00E+00	0.000	N/A		0.00
Leu	133	N-HN	128.386	9.107	6	1.82E+07	0.178	128.431	9.11	6.48E+07	0.309	0.01	1.73	
Thr	145	N-HN	112.076	8.013	68	1.22E+08	1.196	112.04	8.014	1.73E+08	0.824	0.00	0.69	
Phe	155	N-HN	116.329	7.039	18	6.98E+07	0.684	116.409	7.049	1.09E+08	0.519	0.01	0.76	
Ala	159	N-HN	128.626	9.23	9	2.61E+07	0.256	128.597	9.224	7.29E+07	0.347	0.01	1.36	
Gly	164	N-HN	111.895	8.525	10	6.26E+06	0.061	N/A		0.00E+00	0.000	N/A		0.00
Gly	168	N-HN	109.573	8.59	16	6.31E+07	0.619	109.568	8.579	5.93E+07	0.282	0.01	0.46	
Thr	169	N-HN	113.034	7.937	13	4.14E+07	0.406	113.708	7.996	3.22E+06	0.015	0.09	0.04	
Gly	174	N-HN	103.769	8.956	11	3.73E+07	0.366	103.781	8.956	4.48E+07	0.213	0.00	0.58	
Arg	175	N-HN	118.074	8.852	7	1.41E+07	0.138	118.281	8.858	5.08E+07	0.242	0.02	1.75	
Trp	179	N ε 1-H ε 1	130.423	10.236	47	1.02E+08	1.000	130.422	10.234	2.10E+08	1.000	0.00	1.00	
Thr	181	N-HN	107.639	7.711	16	7.15E+07	0.701	107.639	7.711	1.35E+08	0.643	0.00	0.92	
Phe	184	N-HN	118.594	7.039	22	4.71E+07	0.462	118.571	7.044	1.36E+08	0.648	0.01	1.40	
Asp	186	N-HN	114.589	8.681	27	4.84E+07	0.475	114.599	8.684	7.90E+07	0.376	0.00	0.79	
Thr	188	N-HN	115.246	9.524	12	2.99E+07	0.293	115.275	9.52	6.28E+07	0.299	0.00	1.02	
Gly	194	N-HN	108.544	7.868	20	5.71E+07	0.560	108.544	7.868	1.74E+08	0.829	0.00	1.48	
Trp	199	N ε 1-H ε 1	131.278	10.578	26	5.65E+07	0.554	131.243	10.576	1.42E+08	0.676	0.00	1.22	
Gln	200	N ε 2-H ε 2	110.682	7.205	33	7.02E+07	0.688	110.685	7.2	1.49E+08	0.710	0.01	1.03	
Gly	211	N-HN	110.418	8.083	63	1.07E+08	1.049	110.392	8.079	2.76E+08	1.314	0.00	1.25	
Arg	215	N-HN	127.126	7.846	11	4.18E+07	0.410	127.27	7.843	4.74E+07	0.226	0.01	0.55	

Table S5. Crystallographic data collection and refinement statistics of 43/HCMV C-A co-crystal structure, related to Figure 3. The statistics for structure determination are included below.

Data collection	
Wavelength	1.11583
Space Group	P 4 ₁ 2 2
Cell: a, b, c (Å)	51.7, 51.7, 215.7
Resolution (Å) ¹	51.67 – 2.669 (2.766 - 2.67)
Total reflections	212,846 (21,494)
Unique reflections	9,026 (871)
R _{merge}	0.2958 (3.171)
CC _{1/2} ²	0.997 (0.814)
I/sigma(I)	7.94 (1.24)
Completeness (%)	99.35 (98.28)
Redundancy	23.6 (24.7)
 Refinement	
No. reflections	8,969
R _{work} / R _{free} ³	0.248 / 0.288
 Number of atoms	
Protein	213
Ligands	27
Water	4
 B-factors	
Protein	64.32s
 Geometries	
Bond lengths (Å)	0.009
Bond angles (°)	1.22
Ramachandran favored (%)	95.61
Clashscore	14.52

1. Highest resolution shell is shown in parenthesis

2. CC_{1/2} = percentage of correlation between intensities from half data sets.

Resolution limits were calculated using CC_{1/2} > 0.3.

R-factor = S(|F_{obs}| - k|F_{calc}|)/S |F_{obs}| and R-free is the R value for a test set of reflections consisting of a random 5% of the diffraction data not used in refinement.